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Phenolic content and antifungal activity of extracts from *Acacia salicina* L.

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Abstract

This work aims to evaluate the total phenols content and the antifungal activity of different extracts of *A. salicina*. The work was carried out on ethanol and hexane extracts of the bark, flowers and leaves of *Acacia salicina*. Total phenols content was determined. Antifungal activity was tested against four strains; *Alternaria alterna*, *Fusarium culmorum*, *Fusarium oxysporum* and *Fusarium solani*. Ethanol extract from leaves exhibited the highest phenols content (0.74g GAE/ml). The lowest levels were recorded for hexane extract from leaves (0.3g GAE/ml). Statistical analyzes have shown that there is a highly significant difference between ethanolic and hexane extracts. *Fusarium oxysporum* was the most sensitive strain against *A. salicina* extracts.

Keywords:Acaciasalicina, Extracts, Phenols, Antifungal activity.

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1. Introduction

Acacia is an evergreen species that is native to Australia; it has become widely distributed in the Mediterranean region as an introduced species. The genus *Acacia* has 700 species, the best known are *A. cyanophylla*, *A. salicina*, *A. trobilis* and *A. nilotica*.

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Many scientists conducted research on biochemical properties of *Acacia* species. According to their findings, about 152 chemical constituents were isolated from the genus *Acacia*. The most important are flavonoids, terpenoids, phytosterols, phenolic acids, fatty acids, hydrocarbons and others compounds [1,2,3,4]. The biological properties of *Acacia* species have long been studied. Extracts from this species showed significant antimicrobial, antioxidant, antimalarial, anticancer, antidiabetic and anti-inflammatory effects [5,6,7,8]. In traditional Tunisian medicine, the use of *Acacia* differs according to the species and according to the region of the country. Based on information gathered from traditional healers, herbalists, and rural dwellers, *Acacia salicina* is frequently used in diverse applications. This herb is used for the treatment of inflammatory diseases, as a febrifuge, to treat cancer, and to promote human fertility. The fresh chopped leaves are applied directly to the wounds as an anti-inflammatory agent. The traditional and medicinal uses of *Acacia* in northern Tunisia are different. In this region *Acacia* is used for the treatment of diarrhea and rheumatism.

Despite these widespread uses in traditional medicine, works on the biological properties of *Acacia salicina* are limited. This work aims to evaluate the total phenols content and the antifungal activity of different extracts of *A. salicina*.

2. Materials and methods

2.1. Plant material

The work was carried out on the bark, flowers and leaves of *Acacia salicina* collected in the National Institute for Research in Rural Engineering, Water and Forests (INRGREF). The different parts were dried in the oven at 30°C and then powdered using an electric grinder.

2.2. Preparation of the extracts

The hexane and ethanol extracts were prepared by macerating 20 g of powder of the plant material in 200 ml of the solvents for 24 hours. The solvent was evaporated and the dry extracts were collected, weighed and then resuspended in 50 ml of the solvents.

2.3. Extract yield

The percentage of dry crude extract of hexane and ethanol was calculated according to the following formula:

$$R\ (\%) = M / M_0 \times 100$$

R (%): Yield expressed in%; M: Mass in grams of the resulting dry extract and M_0: Mass in grams of the plant material.

2.4. Total phenols contents

Total phenols were determined by the method described by Singleton and Rossi[9]. 150 µl of each sample was mixed with 500 µl of Folin Ciocalteu reagent (1:10 v/v distilled water) and 2 ml of aqueous Na_2CO_3 (2%). The mixtures were allowed to stand for 30 min and the total phenols were determined by colorimetry at 755 nm. The standard curve was prepared using 0, 0.03, 0.06, 0.12, 0.25, 0.5 g/L solutions of gallic acid in water. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

2.5. Antifungal activity

The antifungal activity of *Acacia salicina* extracts was tested against four phytopathogenic fungal strains: *Alternaria alterna*, *Fusarium culmorum*, *Fusarium oxysporum* and *Fusarium solani*. These strains were provided by the National Agricultural Research Institute of Tunisia (INRAT).

The antifungal activity was carried out on a Potato Dextrose Agar medium (PDA). The extracts were added to the medium so as to obtain 100 mg of extract per 20 ml of medium per petri dish.

After cooling the medium, a 5 mm diameter disc of each fungal strain was placed in the center of the petri dish while placing the mycelial surface down. The dishes were incubated at 22 ° C for six days. The fungicidal effect was determined by calculating the growth diameter of the strain and comparing it to that of a negative control, ie PDA medium without extract and containing the appropriate solvent [10]. The results are calculated according to the method of Singh et al. (1993) while calculating the percentage of inhibition according to the following formula:

$$I\ (\%) = [(dC - dE) / dC] \times 100$$

Were: dC: diameter of the negative control (mm) and dE: diameter in the presence of the tested extract (mm).

2.6. Statistical analysis

Statistical processing of data was performed using the SAS General Linear Models (GLM) procedure. An analysis of variance relating to the parameters studied was carried out. The most significant correlations between the latter are noted. The results obtained are presented as the mean of three replicates ± standard deviation.

3. Results and discussion

3.1. Extract yield

Results of the yields of ethanol and hexane extracts from the leaves, bark and flowers of *A.salicina* are shown in Table1.

Table1. Extract yield of *A. salicina*

Plant part	Ethanol	Hexane
Flower	91.83±1.77	26.86±1.17
Leaves	53.5±2.96	8.5±2.5
Bark	47.5±1.27	38.6±3.3

Without realizing the plant material, the best yields were obtained for all ethanolic extracts. The highest yield was achieved by flowers with a percentage of around 91.83%, followed by that of leaves (53.5%) and bark (47.5%).

Using hexane as a solvent, the highest yield was recorded for the bark (38.6%), followed by that of the flowers (26.86%).

The two solvents used during this experiment are of different polarity; ethanol being a polar solvent, while hexane is rather nonpolar.

The significant differences recorded between the yields can be explained by the differential solubility of the different compounds in the solvents used, this being in relation to its polarity [11].

3.2. Total phenols content

The values of the total polyphenol content are summarized in Table2and are expressed in g of gallic acid equivalent per ml of extract (g GAE/ml).

Results showed that the ethanolic extracts are richer in phenols than the hexane extracts. Statistical analyzes have shown that there is a highly significant difference between the two solvents (α <0.0001).

For the ethanolic extract, the leaves showed the highest phenols content with an amount of 0.74g GAE/ml. The lowest levels were recorded for bark (0.68g GAE/ml) and flowers (0.56g GAE/ml).

For hexane extracts, the highest content was recorded for the flowers (0.67g GAE/ml), followed by the bark (0.47g GAE/ml) and the leaves (0.3g GAE/ml).

These results clearly showed that the phenolic compounds are present in the different parts of *Acacia salicina* with concentrations that differ depending on the part of this plant and the solvent used in the extraction.

A study by Bonilla and Amaral Sobra[12]found that the variation in the concentration of total phenolic acids for the same plant largely depends on the solvent used in the extraction. Thus, Clémentine et al. [13]have shown its better to use ethanol as solvent for phenols extraction. It properly solubilizes polar phenolic compounds and can also lead to residual lipophilic substances. Addition of hexane to the extraction system improves the yield of glycosylated phenolics and phenols with a higher degree of polymerization. This partly explains and confirms the results we obtained in our present study.

Phenols have long been known for its antioxidant properties. Several studies reported that diets rich in plant polyphenols protect against cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases [14,15].

Table2. Total phenols content of the ethanol and hexane extracts of the different partss of *A. salicina*(g GAE/ml).

Plant part	Ethanol	Hexane
Flower	0.56 ±0.005	0.67±0.006
Leaves	0.74±0.003	0.3±0.046
Bark	0.68±0.18	0.47±0.013

3.3. Antifungal activity

Results of antifungal activity are illustrated in Figure1.The results showed that the ethanolic extracts exhibited a significant inhibitory activity on all the strains with values higher than those obtained for the hexane extracts. Statistical analyzes have shown that there is a highly significant difference between ethanolic and hexane extracts ($p<0.0001$). The difference between the antifungal activities of hexane and ethanolic extracts may be due to the difference in its chemical composition. Work developed by Traoré et al. [16]. confirms the effectiveness of ethanolic extracts compared to others obtained using other solvents.

Tests against *Fusarium oxysporum* strain showed significant antifungal activity, particularly for the ethanolic and hexane extracts of the leaves which inhibited more than 50% of the mycelial growth of the strain. In contrast, the lowest value was obtained for hexane bark extract (approximately 10% of inhibition).

Many chemical control methods have been tried against *Fusarium oxysporum*. Repeated use of synthetic products such as benomyl, captafol, methylt, the pesticide AJ1629-34EC and others, have given limited results in the medium and long term. They often lead to environmental pollution[17,18,19].The extracts of *A. salicina*, having a significant inhibitory power on this strain, can be used for the control of this phytopathogen.

In the case of *Fusarium culmorum*, the hexane extract of the leaves showed the highest inhibitory effect, resulting in a percent of inhibition of 65.63%. In contrast, the hexane extract from the bark showed the lowest percent inhibition (12.50%). For the ethanolic extracts, it extracts from leaves showed the highest antifungal activity and inhibited the growth of the strain with a percentage of 37%.

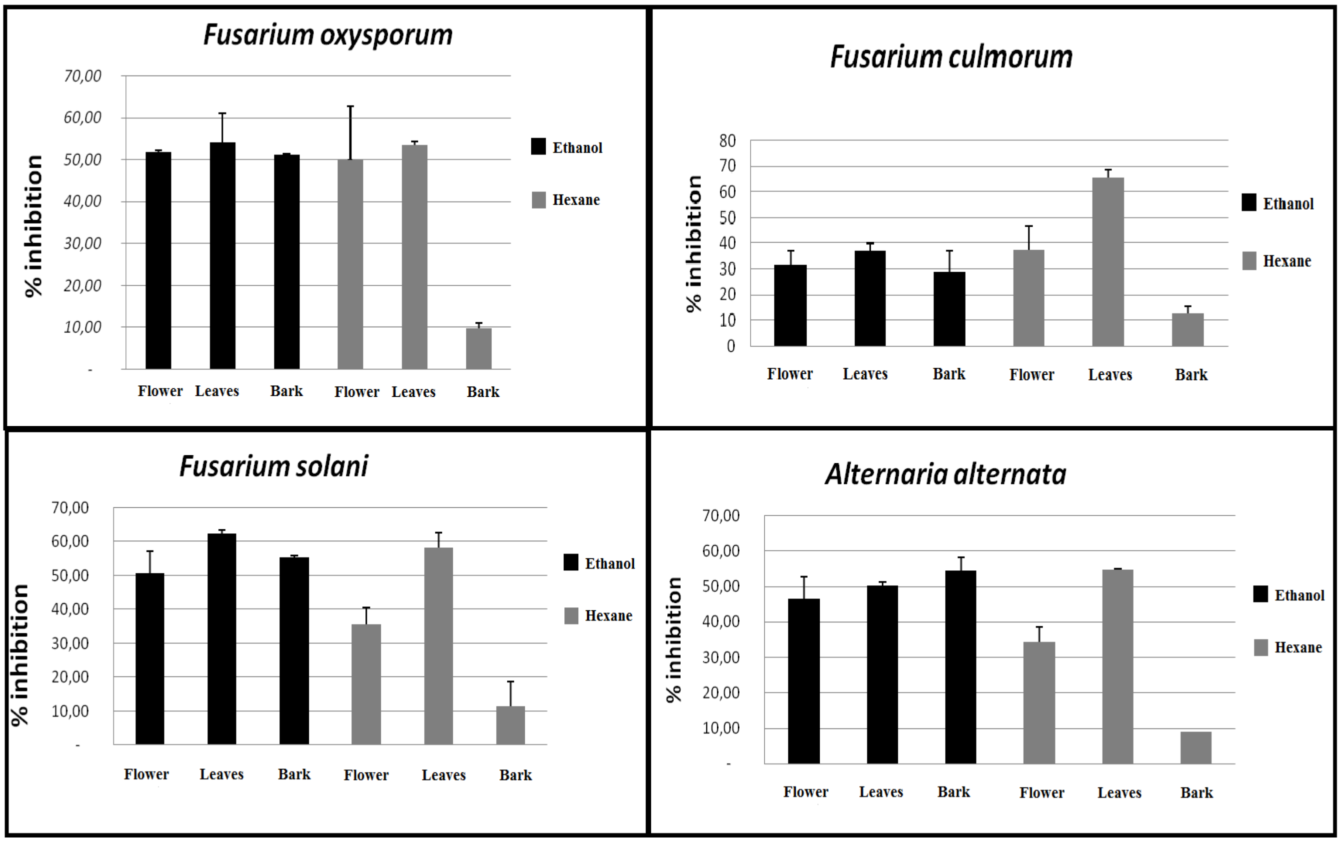

Figure 1.inhibitory activity of studied plants

The inhibitory effect revealed on the *Fusarium solani* strain was clearly higher for the ethanolic leaf extract (62.34%). However, the hexane extract of the bark showed the lowest antifungal activity (11.30%).The fungal strain *Alternaria alternata* was more sensitive in the presence of ethanolic bark and hexane leaves extracts. These two extracts were able to inhibit more than 50% of the mycelial growth of this strain. The lowest antifungal effect was achieved in the presence of hexane bark extract exhibiting a percent inhibition of 9.10%.According to litterature, the varying antifungal effect of the extracts studied may be related to their different compositions. A study developed by Boubaker et al [20]demonstrated that *Acacia salicina* extracts contain active molecules such as flavonoids, polyphenols and coumarins. In addition, a study by Getachew [21]showed that among *Acacia* species, *A. salicina* has been described as a species rich in tannins. The families of these molecules are reported to play an important role in inhibiting the growth of fungi [22].

Therefore, the efficacy of these substances evaluated *in vitro* showed an inhibitory action on microorganisms [23]. Numerous studies have revealed the relationship between the chemical structure of phenolic compounds and their antimicrobial power. Chabot et al. [24]reported that flavonoids which are characterized by the absence of the hydroxyl group on the B ring exhibit higher antimicrobial activity than those with the-OH group.

Consequently, we can conclude that the antimicrobial activity of the extracts depends not only on the phenolic compounds but also on the presence of various secondary metabolites with antifungal effect such as steroids and saponosides which have been reported by several authors [25,26,27].

Conclusion

This work aimed to enhance *Acacia salicina* through the determination of the total polyphenol content as well as the antifungal activity of the extracts of leaves, flowers and bark of this species.

The results of this study support the idea that *Acacia salicina* is an important source of natural antioxidants. In addition, this plant has shown significant antifungal activity and it contains a high content of phenolic compounds.These results can lead to a better valorization of the different parts of this plant and increase the chance of its use in different fields such as biological control of phytopathogens, medicine and pharmacy.

Conflict of Interest-The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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